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Cerebral venous thrombosis post BNT162b2 mRNA SARS-CoV-2 vaccination: A black swan event

To the Editor:

Covid-19 vaccine associated cerebral venous thrombosis (CVT) are rare adverse events and can be considered as *black swan events*. "Black swan events", originally described by Nassim N. Taleb in economic theory, are rare, unexpected events whose potential to occur only becomes apparent after they have happened, where they are neither predicted nor anticipated, and only on retrospect, a clear basis is found for their occurrence that justifies their apparent randomness.

The modified Adenovirus vector Covid-19 vaccines (ChAdOx1 nCoV-19 (Oxford–AstraZeneca) and Ad26.COV2.S (Johnson & Johnson/Janssen)) and mRNA based Covid-19 vaccines (BNT162b2 mRNA (Pfizer–BioNTech) and mRNA-1273 (Moderna)) have shown both safety and efficacy against Covid-19 in phase three clinical trials and are now being utilized in global vaccination programmes. However, as CVT is a rare neurovascular thrombotic event with an estimated incidence of 3 to 4 cases per million per year in unselected populations,[1] clinical trials were underpowered to detect the incidence of CVT. As such, rare cases of post-vaccine associated CVT from the use of ChAdOx1 nCoV-19 and Ad26.COV2.S COVID-19 vaccines, including the mechanism of vaccine-induced thrombotic thrombocytopenia (VITT), have emerged in real-world vaccination with post-marketing surveillance, triggering a wave of temporal suspension of use in several European countries.

The incidence and pathogenesis of CVT after mRNA Covid-19 vaccines remains unknown. Mass vaccination in Singapore commenced on December 30, 2020 with majority of people receiving the BNT162b2 mRNA vaccine, with mRNA-1273 vaccination initiated later on March 12, 2021. We present three cases of CVT post BNT162b2 mRNA SARS-CoV-2 vaccination (Table 1), review existing data on the incidence and discuss mechanisms of CVT in BNT162b2 mRNA vaccination.

Patient 1: A healthy 54-year-old Chinese male with well controlled hyperlipidaemia, developed severe headache and vomiting 24 h after his second dose of BNT162b2 mRNA vaccine, and acute left hemiparesis 2 days later. A CT Brain revealed a large right temporo-parietal lobe intraparenchymal hemorrhage with associated midline shift and uncal herniation, necessitating a decompressive craniectomy. A CT angiogram excluded underlying vascular malformations, and CT venogram confirmed dural venous sinus thrombosis (transverse and sigmoid sinus). Full blood count, coagulation profile and thrombophilia screen were unremarkable. Tests for anti-platelet factor 4 (anti-PF4) antibodies and heparin induced platelet aggregation (HIPA) were negative. Bilateral lower limb deep vein thrombosis ultrasound scans were negative. Whole body CT did not reveal any other thrombosis or malignancy. He was anticoagulated on unfractionated heparin (UFH) and subsequently converted to low molecular weight heparin (LMWH) and is currently undergoing rehabilitation.

Patient 2: A healthy 62-year-old Chinese female with a history of well-controlled hypertension presented with headache and vomiting 9 days after her second dose of BNT162b2 mRNA Covid-19 vaccine. Her full blood count and coagulation panel were unremarkable. Both CT Brain and CT venogram on admission confirmed acute right cerebral bleed involving occipital and temporal lobes associated with subarachnoid hemorrhage due to thrombosed right transverse and sigmoid sinus veins. Unfractionated heparin was started with close therapeutic monitoring. On day 4 of admission there was a drop in her Glasgow Coma Scale from 14 to 9, with repeat CT showed increasing size of haemorrhagic right cerebral venous infarcts with worsening of mass effect and development of early hydrocephalus, requiring decompressive craniectomy. This was later complicated by intracranial empyema requiring drainage. Post operatively, UFH infusion was resumed and later converted to LMWH. Thrombophilia workup was negative. Bilateral lower limb deep vein thrombosis scan was negative for thrombosis. Whole body CT performed 3 weeks into admission was negative for malignancy, however, right upper lobe segmental artery pulmonary embolism, left internal iliac artery and right common iliac vein thrombi were detected, likely from immobility in ICU and perioperative discontinuation of anticoagulation. Incidentally, a left iliopsoas haematoma was detected. Despite her haematoma, UFH was resumed due the increased thrombotic burden and later converted to LMWH, with bridging to warfarin a week later.

Patient 3: A 60-year-old Chinese female with family history of thrombosis (her son had unprovoked pulmonary embolism) and medical history of diabetes mellitus, hypertension and hyperlipidaemia, presented 8 days after her second dose of BNT162b2 mRNA Covid-19 vaccine for right ataxic hemiparesis. Both CT brain and venogram confirmed extensive dural venous thrombosis and venous infarct in bilateral perirolandic gyri. This was complicated by acute right occipital lobe intraparenchymal hematoma and bilateral subarachnoid hemorrhage. Her CVT was treated with LMWH, followed by bridging to warfarin. Thrombophilia workup apart from a low anti thrombin III (AT) level of 55% (reference range 80%–130%), was unremarkable. She had no previous history of deep vein thrombosis. Her low levels of AT III during acute illness could possibly be attributed to acute and extensive thrombosis which can lower levels transiently, hence plans were made to recheck AT III levels when the patient is asymptomatic. Whole body CT scan performed did not reveal any malignancy. She made an uneventful recovery and was discharged.

Platelet flow cytometry (Becton Dickinson FACSCanto TM Flow analyzer) performed on blood specimens from patient one and two both demonstrated increased expression of CD62P (platelet surface P-selectin) and PAC1 (activated GP IIb/IIIa) in the presence of an ADP

TABLE 1 Clinical characteristics, neuroimaging, laboratory tests and outcomes of the three patients[a]

Patient number	1	2	3
Clinical Characteristics			
Age (years)	54	62	60
Gender	Male	Female	Female
Past medical history	Hyperlipidemia	Hypertension	Diabetes mellitus Hypertension Hyperlipidemia
Number of vaccine doses	2	2	2
Onset of symptoms post vaccination (days)	1	9	8
Presenting symptoms	Headache followed by left sided weakness	Headache, vomiting, behavioral changes	Giddiness, vomiting and right sided weakness and numbness
Neuroimaging			
Site of thrombosis	Right transverse sinus Right sigmoid sinus Right internal jugular veins	Right transverse sinus Right sigmoid sinus Right internal jugular veins	Right transverse sinus Right sigmoid sinus Right internal jugular veins Bilateral cortical veins
Hemorrhage	Right temporo-parietal intraparenchymal hemorrhage	Right temporal-occipital hemorrhage and subarachnoid hemorrhage (SAH)	Right occipital hematoma, bilateral SAH and bilateral peri-rolandic gyri infarcts
Laboratory Tests			
White blood count ($x10^9$/L)	12.9 (4.0–9.6)	18.79 (3.82–9.91)	7.34 (4.0–10.0)
Hemoglobin (g/dL)	13.5 (13.6–16.6)	12.8 (11.2–14.9)	13.5 (12–16)
Platelets ($x10^9$/L)	300 (150–360)	383 (173–414)	346 (140–440)
Prothrombin time (sec)	13.9 (11.7–14.0)	10.4 (9.7–11.6)	10.3 (9.9–11.4)
Activated partial thromboplastin time (sec)	28.7 (27.0–37.0)	22.6 (25.5–35.8)	28.2 (25.7–32.9)
INR	1.0	0.96	0.98
ESR (mm/hr)	35 (1–10)	50 (1–20)	-
ANA (titre)	<80 (<80)	0.07 (<1.0)	-
Anti-dsDNA (titre)	<25 (0–25)	57.1 (<100)	-
Anti-Cardiolipin IgM	Negative	Negative	Negative
Anti-Cardiolipin IgG	Negative	Negative	Negative
Lupus anticoagulant	Absent	Absent	-
Anti B2 glycoprotein 1	Negative	Negative	-
Factor V levels (%)	-	134 (64–161%)	-
Factor VIII levels (%)	-	>200 (53–196%)	-
Anti-thrombin III (%)	94 (80–130)	87 (93–125)	55 (80–120)
Protein C (%)	87 (70–150)	146 (83–144)	177 (70–140)
Protein S (%)	76 (65–130)	99 (65–115)	69 (64–123)
Factor V Leiden gene	-	Negative	-
Anti-PF4 antibody	Negative	-	-
Heparin induced platelet aggregation	Negative	Negative	-
Platelet flow cytometry	Increased CD62P and PAC1 expression	Increased CD62P and PAC1 expression	Not performed
Treatment and outcome			
Anticoagulation	UFH followed by LMWH then warfarin	UFH followed by LMWH then warfarin	LMWH then warfarin
Clinical outcome	Left hemiparesis, on rehabilitation	Left hemiparesis, on rehabilitation	Full recovery

[a]Reference ranges differ as patients were admitted to three different hospitals.

agonist), with normal expression of CD36, CD42a, CD42b, CD61, Cd63, GPVI and CD235a as compared to healthy controls.

Emerging data from pharmacovigilance databases and case reports discuss the rare incidence of CVT post BNT162b2 mRNA vaccination. Smadja et al.[2] evaluated Covid-19 vaccination thrombotic risk reported to the World Health Organization (WHO) Global Database for Individual Case Safety Reports (VigiBase), a global pharmacovigilance database monitoring safety of ChAdOx1 nCoV-19, BNT162b2 mRNA and mRNA-1273 vaccines. For the three vaccines, 2169 thrombotic events (795 venous and 1374 arterial thrombotic events) were reported from December 12, 2020 to March 16, 2021, with a combined reporting rate of 0.21 [95% CI: 0.19–0.22] cases of thrombotic events per 1 million person vaccinated-days. The reporting rate of unexpected CVT post Covid-19 vaccine for mRNA-1273 (Moderna) was 0.9%, with BNT162b2 mRNA (Pfizer–BioNTech) was 0.4%, and for ChAdOx1 nCoV-19 was 1.1%. Overall median time to venous thrombotic event was 4 days. A caveat for this dataset is that incidence or prevalence are not appropriate since there is no information about precise denominator for each separate vaccine and about the extent of underreporting.

In the Yellow Card report[3] covering the period of December 9, 2020 to May 26, 2021, the Medicines and Healthcare products Regulatory Agency in the United Kingdom noted 33 cerebrovascular venous and sinus thrombosis (24 cerebral venous sinus thrombosis, three CVT and three superior sagittal sinus thrombosis, one transverse sinus thrombosis) out of a total of 10.6 million second doses of BNT162b2 mRNA vaccine given, or 33/10 600 000 (0.00311%).

There has only been a single published case report of two female patients with extensive CVT post BNT162b2 mRNA vaccination by Dias et al.[4] Both patients had normal platelet counts and negative tests for anti-PF4 antibodies, excluding VITT. The first patient had borderline low protein S, possibly depressed due to extensive thrombosis. The relationship of CVT to BNT162b2 remains uncertain given the patients' pre-existing risk factors of oral contraceptives use and renal cell carcinoma.

As of May 31, 2021, 4 047 651 million doses of mRNA vaccines have been administered in Singapore, with three cases of possible Covid-19 vaccine associated CVT, deriving an incidence of 3/4 047 651 (0.000074%) per dose, or 3/1 766 497 (0.00017%) for people who have completed two vaccination doses, as compared to a higher incidence of 2/44 479 (0.0045%) of CVT associated with Covid-19 infection in a local review.[5] In our three cases described, patients affected were predominantly older, with development of CVT on the first to the ninth day after the second dose of BNT162b2 mRNA vaccination. All had extensive CVT, with the first and second patient developing life threatening intraparenchymal hemorrhage requiring decompression craniectomy. All had normal platelet counts, with two patients having negative HIPA, suggestive that the mechanism of CVT was unrelated to VITT described with the modified Adenovirus vector Covid-19 vaccines. Therefore, anticoagulation with LMWH or UFH is appropriate and should not be avoided in mRNA Covid-19 vaccine associated CVT.

Covid-19 has strong association with immunothrombosis, resulting in stroke and CVT.[5] So, CVT occurring post Covid-19 mRNA vaccination could be a possible recapitulation of the thrombotic complications seen in acute Covid-19, where the WHO has cautioned that "Covid-19 vaccination itself may be associated with an increased risk of developing Covid-19-like disease or its complications[6]". While the SARS-CoV-2 virus has a higher thrombogenic potential, the spike glycoprotein also carries significant thrombogenicity. We therefore postulate that the pathogenesis of BNT162b2 mRNA vaccine induced CVT may possibly be related to spike glycoprotein interaction causing immunothrombosis from 1) Disruption of endothelial homeostasis and the blood–brain barrier 2) SARS-CoV-2 spike glycoprotein directly causing platelet activation 3) Aberrant activation of the alternative pathway of complement.

Firstly, evidence for endothelial dysfunction is demonstrated in a 3-D microfluidic model of the human blood–brain barrier (BBB),[7] where spike glycoprotein, upon binding to the angiotensin converting enzyme 2 receptor, induces loss of the BBB integrity, activation of endothelial cells with upregulation of cell adhesion molecules (ICAM-1 and VCAM-1), leukocyte chemotaxis factors and proinflammatory cytokines (IL-1 β and IL-6), triggering a proinflammatory response and upregulation of matrix metalloproteinases in human brain endothelial cells. We postulate that this mechanism may contribute to a significant breach in brain endothelial barrier integrity. A combination of endothelial dysfunction, exposed tissue factors, immune-vascular cross talk and procoagulant cytokines from vessels can promote thrombogenesis and cause catastrophic CVT.

Secondly, Zhang et al.[8] demonstrate that the spike glycoprotein potentiates platelet aggregation and dense granule secretion in response to various platelet agonists, including ADP, with platelets demonstrating accelerated spreading and clot retraction. Flow cytometry demonstrated an enhancement of both platelet activation markers of integrin αIIbβ3 activation (CD62P) and P-selectin expression (PAC-1) in the presence of agonist, *similar* to the flow cytometry findings in our two patients, which can be attributed to possible prior spike glycoprotein sensitization from mRNA Covid-19 vaccination. Despite clear evidence that vaccine mRNA is degraded within short days[9,10] the duration of persistence of the spike glycoprotein in vivo for the BNT162b2 mRNA vaccine is still unknown, and expert opinion from the Infectious Diseases Society of America vaccines information FAQ postulates that the spike glycoprotein may last for weeks. While platelets can be activated in the immediate post thrombotic state or after acute bleed, post-vaccination persistence of spike glycoprotein in tissues or in the circulation could be a plausible cause for platelet activation, as seen in the unusual persistence of increased expression of platelet activation markers CD62P and PAC1 in the flow cytometry of patient two, performed 4 weeks post neurosurgery.

Thirdly, in vitro studies[11] have demonstrated that spike proteins can activate the alternative pathway. Carvelli et al.[12] report activation of C5a and sC5b-9 as well as deposition of activated complement activation products in injured tissues and organs in severe Covid-19 infection, through activation of any of three initiating pathways (classical, lectin, or alternative pathway) by the spike protein or immune-antigen complexes, with concurrent monocyte activation and neutrophil NETosis. Spike

protein dysregulation of the complement system can perpetuate a vicious cycle of inflammatory tissue damage and cause significant endothelial dysfunction, playing a pivotal role in immune-mediated thrombogenesis.

We propose that a combination of one or more conditions - a sufficiently high enough titre of spike glycoprotein in the circulation, coupled with a high number of activated platelets and aberrant complement activation by the spike glycoprotein - need to be present to cross the thrombotic threshold and trigger CVT. This may explain the rarity of this clinical observation. Further studies are required to determine the causal association of mRNA Covid-19 vaccines and CVT.

In conclusion, robust pharmacovigilance for Covid-19 vaccines is key in advancing global Covid-19 vaccination efforts with both speed and safety. However rare they may be, the reporting of adverse and *black swan events* such as Covid-19 vaccine-associated CVT is necessary to minimize uncertainty in the medical fraternity and the public, to increase awareness of regulatory authorities and clinicians to such events, so that these can be evaluated and managed promptly. Compared to most medications where adverse reactions that occurs in fewer than 1 in 10 000 are considered as "very rare", Covid-19 vaccines appear safe, given that risk of Covid-19 vaccine-associated CVT is by far much rarer than developing CVT in Covid-19 infection.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

B.E.F., J.Y.S., B.M.R.V.P. conceived the study. All authors were involved in patient care, and contributed substantially in the acquisition, analysis and interpretation of data, critical revision of manuscript for important intellectual content.

ETHICS STATEMENT

The study was approved by the Singapore Health Services institutional review board (CIRB 2020/2410). Waiver of consent was granted by the Singapore Health Services institutional review board.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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